amendments is provided throughout the disclosure and discussed in detail below. Entry of this amendment and reconsideration of the application are respectfully requested.

Applicants note that the rejections under 35 U.S.C. § 112, second paragraph of claims 4 and 6 have not been repeated and, therefore, are deemed waived (MPEP 707.07(e)).

Amendments to the Specification

The Examiner objected to the specification at page 13, in the brief description of Figures 2 and 3, as failing to comply with 27 CFR 1.821 through 1.825. Applicants believe that the specification as filed complies with 27 CFR 1.821 through 1.825, e.g.,1.821(d), as shown by the description of Figures 2 and 3 that appears at page 21, line 14 to page 22, line 1. Nonetheless, to be responsive to the Examiner's objection, Applicants have amended page 13, lines 2-14, consistent with the information that appears at page 21, line 14 to page 22, line 1.

The Examiner objected to the abstract of the disclosure because it is less than 50 words and required correction, citing MPEP § 608.01(b), which cites 37 CFR 1.72(b):

> A brief abstract of the technical disclosure in the specification must commence on a separate sheet, preferably following the claims, under the heading "Abstract" or "Abstract of the Disclosure." The abstract in the application filed under 35 U.S.C. 111 may not exceed 150 words in length. The purpose of the abstract is to enable the United States Patent and Trademark Office and the public generally to determine quickly from a cursory inspection the nature and gist of the technical disclosure. The abstract will not be used for interpreting the scope of the claims.

Applicants were unable to find any minimum required number of words for the abstract in 37 CFR 1.72(b), although MPEP § 608.01(b) states that the abstract "should be generally limited to a single paragraph within the range of 50 to 150 words." The Examiner also provided some information on phrases (related to "disclosure") to be avoided in the abstract. Applicants believe that the 47-word abstract as filed meets the requirements of 37 CFR 1.72(b) and is grammatically correct ("disclosed" is the verb in both sentences). Nonetheless, to be responsive to this objection, Applicants have amended

the abstract, adding a total of 21 words, deleting a total of 6 words (including "disclosed"), for a net addition of 15 words, bringing the abstract to a total of 62 words. If the Examiner finds the amended abstract to be unacceptable, Applicants request that the Examiner either provide additional clarification of the objection (i.e., suggest specific changes) or change the abstract by an Examiner's amendment as provided for in MPEP § 608.01(b).

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 21-26 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite, particularly based on a typographical error that appears in claim 21 in the "contacting" step (b).

Applicants thank the Examiner for her careful reading of claim 21 and regret the confusion caused by the typographical errors that appeared in the claim. Claim 21 has been amended in the "contacting" step (b) to simplify the claim by removing some functional language in the contacting step (b), inserting the terms "*abl*-specific" and "*bcr*-specific" to provide clarity when referring to the primers, and inserting SEQ ID Nos for structures used in the "contacting" step (b). Based on this simplification to step (b), the "amplifying" step (c) has also been simplified by using similar terms as in step b, and the "hybridizing" step (d) has been clarified by referring to a "*bcr*-specific" probe.

Claim 22 has been similarly amended by removing functional language in the "contacting" step clause, inserting the clarifying term "an *abl*-specific" for the third primer, and inserting a SEQ ID NO. In the "amplifying" clause, some functional language has been deleted in favor of referring to "using the *abl*-specific first primer and the *abl*-specific third primer".

Based on these amendments, Applicants respectfully request allowance of claims 21-26.

Rejections under 35 U.S.C. § 112, First Paragraph

Claims 21-26 stand rejected under 35 U.S.C. § 112, first paragraph. Because of the typographic errors in claim 21, the interpretation of the claim was different than Applicants intended and the Examiner reasonably questioned the support for the amended claims as required by 35 U.S.C. 112, first paragraph. Although Applicants pointed out sections of the specification in which support for the claims

is provided, those sections did not appear to support the claims due to the typographical errors in claim 21 that resulted in an unintended interpretation of the claims. Applicants again apologize for the confusion caused by the typographical errors, and respectfully point out the support for claims 21-26 in the following summary.

The specification describes embodiments claimed in amended claim 21 in the drawings of Figures 1A and 1B, 2 and 3, and the descriptions that appear at page 21, line 14 to page 22, line 1. Figures 1A and 1B schematically show two different *bcr-abl* fusion sequences and the relative positions of the *abl*-specific and *bcr*-specific primers, and the *bcr*-specific probe relative to the different splice junctions of the fusion sequences. Figures 2 and 3 show structural sequence information that is related to the schematic drawings of embodiments shown in Figures 1A and 1B. Figure 2 shows the *abl*-specific sequence of SEQ ID NO:22 embedded within the entire sequence shown (SEQ ID NO:25), as described at page 21, lines 20-22. SEQ ID NO:22 is a primer binding site for the *abl*-specific portion of SEQ ID NO:1; the complementarity of these sequences can be readily determined by one skilled in the art merely by comparing the sequences. Figure 2 also identifies the *bcr*-specific sequences of SEQ ID NO:5 and SEQ ID NO:9 as part of the entire sequence (see page 21, lines 16-19). Figure 3 similarly shows one embodiment sequence and identifies the sequences embedded within it.

The specification describes embodiments of amended claim 22 in the drawing of Figure 1C and the description that appears at page 19, line 25 to page 21, line 13. Figure 1C schematically shows a normal *abl* sequence, the relative positions of two *abl*-specific primers, and an *abl*-specific probe relative to a position in the *abl* sequence where a splice junction may occur in a *bcr-abl* fusion sequence. Figure 1C represents normal (unspliced) *abl* sequence which may serve as an internal control (see page 21, lines 8-13).

Embodiments of claims 21- 26 are further illustrated by Examples 2 to 6. Example 2 (page 27, line 25 to page 29, line 12) describes an embodiment that uses an *abl*-specific first primer of SEQ ID NO:1 (page 28, lines 3-4), which contains a sequence complementary to SEQ ID NO:22, as can be

readily determined by one skilled in the art by sequence comparision. Example 2 also describes using a *bcr*-specific second primer of SEQ ID NO:5 (page 28, lines 5-6) with the first primer in an amplification reaction (page 28, lines 10-15) that uses an enzyme having an RNA-directed DNA polymerase activity (MMLV reverse transcriptase) and an enzyme having an DNA-directed RNA polymerase activity (T7 RNA polymerase). Example 2 describes detection of amplified RNA using a *bcr*-specific probe of SEQ ID NO:9 (page 28, line 16 to page 29, line 6).

Example 3 (page 29, line 13 to page 30, line 17) describes an embodiment that also includes amplification and detection of a normal *abl* sequence to serve as an internal control. For amplification of a *bcr-abl* fusion sequence, the *abl*-specific first primer is of SEQ ID NO:1 and the *bcr*-specific second primer is of SEQ ID NO:5 (page 29, lines 21-22). The internal control was amplified using the *abl*-specific first primer is of SEQ ID NO:1 and the *abl*-specific third primer is of SEQ ID NO:13 (page 29, lines 21-24, and page 30, lines 4-7). Detection of the amplified sequences is described by using a *bcr*-specific probe of SEQ ID NO:9 (page 29, lines 25-29) and an *abl*-specific probe of SEQ ID NO:16 (page 29, line 29 to page 30, line 7).

Example 4 (page 30, line 19 to page 32, line 13) describes an embodiment that uses an *abl*-specific primer of SEQ ID NO:1 (page 31, lines 8-11), a *bcr*-specific probe of SEQ ID NO:9 (page 31, line 15), and an *abl*-specific probe of SEQ ID NO:16 (page 31, line 16).

Example 5 (page 32, line 14 to page 33, line 17) describes an embodiment that uses an *abl*-specific primer of SEQ ID NO:1 and an *abl*-specific primer of SEQ ID NO:13 in amplification (page 32, lines 26-28), and an *abl*-specific detection probe of SEQ ID NO:16 (page 32, line 28 to page 33, line 1).

Example 6 (page 33, line 18 to page 35, line 8) describes embodiments in which *bcr-abl* fusion sequences are amplified by using an *abl*-specific first primer of SEQ ID NO:1 and a *bcr*-specific second primer is of SEQ ID NO:5, and detected by using *bcr*-specific probes of SEQ ID NO:9 and SEQ ID NO:27 (page 34, lines 7-10, and Table 3). The normal *abl* sequences served as an internal control and were detected by using an *abl*-specific probe of SEQ ID NO:26 (page 34, lines 10-11, Table 3, and page 35,

lines 5-6).

Applicants respectfully submit that amended claims 21-26 are fully supported by the subject matter described in the specification. The descriptions provided in the specification would reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Therefore, Applicants request that the rejections based on 35 U.S.C. 112, first paragraph be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 1-5 and 9-17 stand rejected under 35 U.S.C. § 102(b) as anticipated by the disclosure of Sooknanan et al. (Experimental Hematology 21: 1719-1724, 1993), for the same reasons of record as set forth in the office action mailed December 21, 2001. To be anticipated, each limitation of a claimed invention must be found in the cited art reference.

Claims 1, 3, 5, 9, 10, and 14 have been amended. Claims 4, 11, and 13 have been canceled. Amended claim 1 specifies that fusion nucleic acid contains a *bcr-abl* splice junction (steps a and c), that the first primer is "*abl*-specific" and hybridizes to a primer binding site of SEQ ID NO:22 (step b), that the probe binding site is located "in a *bcr*-derived sequence" which is not overlapping the complementary splice junction site (step c), and that the hybridizing step uses a *bcr*-specific probe (step d). Because of the insertions of structural language (e.g., SEQ ID NO:22) into claim 1, other language describing the locations 3' or 5' of the splice junction site have been deleted. Amended claim 3 contains structural language ("*bcr*-specific" and "SEQ ID NO:5") and deletes language related to locations 3' of other sites. Claim 5 has been amended to specify a *bcr*-specific probe that has a sequence of SEQ ID NO:9 or SEQ ID NO:27, and by deleting limitations now found in amended claim 1. Amended claim 9 specifies that fusion mRNA transcript contains a *bcr-abl* splice junction (step a), the first primer hybridizes to a sequence of SEQ ID NO:22 (step b), the probe binding site is derived from a *bcr* sequence and not overlapping the complementary splice junction site, and the second probe binding site is derived from an *abl* sequence relative to sequence complementary to SEQ ID NO:22 (step c), and the hybridizing

step forms a hybridization complex with the first probe or the second probe (step d). Because of the insertions of structural language into claim 9, language related to locations 3' or 5' of the splice junction site or chromosomal regions has been deleted. Amended claim 10 includes structural language for SEQ ID NO:1. Amended claim 14 is made dependent on claim 9 and deletes language redundant of amended claim 9. Support for these changes is provided in the specification (e.g., page 21, line 14 to page 22, line 1; page 27, line 25 to page 29, line 12; page 33, line 18 to page 35, line 8; page 33, line 18 to page 35, line 8; page 29, line 13 to page 30, line 17; page 30, line 19 to page 32, line 13; and page 32, line 14 to page 33, line 17) as described above for 35 U.S.C. § 112, first paragraph.

Amended independent claims 1 and 9 contain structural limitations not taught by Sooknanan et al. As discussed in the response filed June 21, 2002, Sooknanan et al. describe detection of BCR-ABL mRNA by using nucleic acid amplification methods that rely on serial amplification reactions using two sets of internal nested oligonucleotide primers, and detection probes specific for BCR-ABL *junctions* (i.e., *spanning* either the bcr3-abl2 *junction* or the bcr2-abl2 *junction*). This is unlike Applicants' claimed methods. As pointed out earlier, Applicants stated in the specification (page 17, lines 20-21.): "It is important to note that this method does not use nested primers and does not require use of serial amplification reactions." Claims 1 and 9 (step c) make clear that the probe binding sites in either a *bcr*-derived sequence or an *abl*-derived sequence are "not overlapping the complementary splice junction site." This is supported in the specification (e.g., page 18, lines 23-24, page 18, lines 27-29, page 19, lines 1-6, page 19, lines 22-24, and page 19, line 29 to page 30, line 3) as discussed in the response filed June 21, 2002. In contrast, Sooknanan et al. use probes that *overlap* the splice junction sites by spanning either the bcr3-abl2 *junction* or the bcr2-abl2 *junction*.

The Office Action incorrectly characterized the embodiment illustrated by Applicants' Figure 1C in the statement "Furthermore, Applicant's invention according to Figure 1C embraces an abl probe that spans the translocation junction" (page 9, lines 11-12). As described in the above section of this response under 35 U.S.C. § 112, first paragraph, Applicants Figure 1C schematically shows a normal *abl*

sequence, and the relative positions of two *abl*-specific primers and an *abl*-specific probe compared to the location in the *abl* sequence where a splice junction may occur if a normal *abl* sequence were to participate in a translocation event that fused a *bcr* and an *abl* sequence. The probe shown in Figure 1C is an *abl*-specific probe (specific for the normal *abl* sequence) which would not function if a *bcr-abl* fusion occurred, because the binding site for the *abl*-specific probe would be functionally destroyed (see page 19, line 25 to page 21, line 13). Figure 1C illustrates a normal (unspliced) *abl* sequence which may serve as an internal control (page 21, lines 8-13). Example 3 describes an internal control embodiment detected by using an *abl*-specific probe (page 29, lines 21-24, and page 29, line 29 to page 30, line 7).

Based on the above arguments, Applicants respectfully submit that Sooknanan et al. do not teach each limitation of the claimed invention. Therefore, Applicants request allowance of amended claims 1-5 and 9-17.

Claims 19 and 20 stand rejected under 35 U.S.C. § 102(a) based on the disclosure of the QIAGEN Oligotex Direct Protocol for isolation of PolyA+ mRNA (QIAGEN product guide, 1/98, page 61, and QIAGEN web page), combined with the disclosures of U.S. Patent Nos. 6,054,294 and 5,837,493 (page 10, section 9). As stated above, a rejection under 35 U.S.C. § 102(a) must rely on the invention described in "a printed publication" (i.e., a single prior art reference; MPEP 2132). The Examiner has cited three references. The first cited reference is the "Oligotex Direct mRNA Protocol" referenced in the 1/98 Qiagen Product Guide. This is not prior art because it was published after Applicants' priority date, as discussed in the response filed June 21, 2002. The second and third cited references ('294 patent at section 144 and '493 patent at sections 156 and 160) were provided because, according to the Office Action, they "teach that the Oligotex protocol was known" at the priority dates of the applications for the '294 and '493 patents. The referenced texts of the '294 and '493 patents merely state that a QIAGEN OLIGOTEX system or kits were used, without describing the system or kits in detail. No cited prior art reference in the Office Action describes the claimed invention. Despite the statement that "Absent evidence to the contrary, the buffers and directions for mRNA isolation were the same in the protocol

provided to the Applicant and the methods described in U.S. Patents '493 and '294 (page 11, lines 1-2), a *prima facie* case under 35 U.S.C. § 102(a) has not been established (MPEP 2132). Therefore, Applicants respectfully request withdrawal of the rejection of claims 19 and 20 under 35 U.S.C. § 102(a).

Rejections under 35 U.S.C. § 103(a)

Claims 6-8 and 18 stand rejected under 35 U.S.C. § 103(a) for the reasons of record in the Office Action mailed December 21, 2001. Rather than repeat the legal bases for establishing a *prima facie* case of obviousness, Applicants respectfully refer the Examiner to the comments that appear in the response filed on June 21, 2002, page 15.

Claims 6-8 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Sooknanan et al. in view of the QIAGEN Oligotex Direct Protocol for isolation of PolyA+ mRNA.

As discussed above, amended claim 1, from which claim 6 depends, includes structural features that Sooknanan et al. do not teach and, in fact, teach away from (e.g., a probe that hybridizes to a sequence "not overlapping the complementary splice junction site"). Therefore, dependent claim 6 cannot be obvious based on the Sooknanan et al. reference.

As discussed above, the Examiner has not established that the cited QIAGEN reference is prior art to Applicants' invention. The '493 and '294 patent references that were improperly cited in the rejection based on § 102(a) were not cited in the rejections under § 103(a). Assuming *arguendo* that the Examiner meant to cite the '493 and '294 references in the § 103(a) rejections, those references provide no specific information on the later published QIAGEN reference. The '493 and '294 references merely show that QIAGEN was providing some kind of undefined system or kits for isolating mRNA as of the priority dates of the '493 and '294 references. It is improper to presume that a trade name of an earlier product defines the components of a later-published reference that uses the same trade name because there is no proof that the two are related in anything except the name. Therefore, the obviousness rejection of claims 6-8 relies solely on the disclosure of Sooknanan et al. For the reasons discussed above regarding amended claim 1, and based on the arguments above, Applicants respectfully submit

that the Examiner has not established a *prima facie* case of obviousness. Therefore, Applicants request that the rejection of claims 6-8 under 35 U.S.C. § 103(a) be withdrawn.

Claim 18 stands rejected under 35 U.S.C. § 103(a) as unpatentable over Sooknanan et al. in view of the QIAGEN Oligotex Direct Protocol for isolation of PolyA+ mRNA and further in view of Burg et al. (US Patent 6,300,068 B1) for the reasons of record as set forth in the office action mailed December 21, 2001.

As discussed above under the section for 35 U.S.C. § 102, claim 9, from which claim 18 depends has been amended to specify that the fusion mRNA transcript contains a *bcr-abl* splice junction (step a), the first primer hybridizes to a sequence of SEQ ID NO:22 (step b), the probe binding site is derived from a *bcr* sequence and is not overlapping the complementary splice junction site, and the second probe binding site is derived from an *abl* sequence (step c), and hybridizing (step d) forms a hybridization complex with the first probe or the second probe. Claim 18 has been amended to refer to a "normal *abl*" transcript and "an *abl*-specific" probe. As discussed above, this is unlike anything the Sooknanan et al. reference teaches or suggests. As discussed above, the Examiner has not established that the QIAGEN reference is prior art. Burg et al. was cited for teaching the use of an internal control transcript in methods of nucleic acid amplification, but this general description of an internal control cannot suggest the invention of dependent claim 18, even if disclosures of Burg et al. and Sooknanan et al. are combined because Sooknanan et al. teach using detection probes that span the splice junctions. The internal control of claim 18 does not include a splice junction, and cannot be suggested by the cited art. Therefore, Applicants respectfully request that amended claim 18 be allowed.

CONCLUSION

In view of the foregoing amendments and remarks, the Applicants respectfully submit that the claims are in condition for allowance. Accordingly, allowance of the application is earnestly solicited. The undersigned has made a good-faith effort to address all the points raised in this Office Action and to

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place the claims in condition for allowance. If minor matters remain that could be resolved by telephone interview, the Examiner is invited to contact the undersigned at the number below.

Applicants believe that no additional fees are due in connection with filing this amendment. If, however, Applicants are mistaken and a fee is due, then Applicants' representative hereby provides authorization that the required fee(s) due in connection with this filing be debited form Deposit Account No. 07-0835.

Certificate of Facsimile Transmission

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is being facsimile transmitted to the United States Patent and Trademark Office, facsimile number 703-872-9307 on the date shown below.

Respectfully submitted,

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Enclosures: Appendix A (marked up specification text)
Appendix B (marked up claims)

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Appendix A

IN THE SPECIFICATION

Please make the following changes as shown underlined in the marked up copy of the text.

At page 13, lines 2-15:

FIG. 2 shows the 5' to 3' DNA base sequence (SEQ ID NO:24) of the region surrounding the *bcr-abl* splice junction, as shown schematically in FIG. 1A, where the underlined region (residues 1 to 126) represents the *bcr* b2 sequence containing the sequence complementary to a primer binding site (bolded residues 65 to 88, SEQ ID NO:5) and the sequence complementary to the b2 probe binding site (bolded and italicized residues 89 to 113, SEQ ID NO:9); the double underlined region (residues 127 to 201) represents the *bcr* b3 sequence; the splice junction occurs between bases 201 and 202 and the remaining sequence is the A2 region of *abl* containing the *abl* primer binding site (bolded, SEQ ID NO:22).

FIG. 3 shows the 5' to 3' DNA base sequence (SEQ ID NO:25) of the region surrounding a potential splice junction in a normal *abl* transcript where: residues 1 to 151 are *abl* 1b exon sequence containing a region complementary to an *abl* primer binding site (residues 84-103, bolded, SEQ ID NO:13); the double-underlined region (residues 102 to 119, SEQ ID NO:26) is the complement of an *abl*-specific probe binding site flanking the splice junction of *abl* b1 and *abl* b2; the underlined region (residues 142 to 165, SEQ ID NO:16) is the complement of second probe binding site that overlaps potential splice junctions; and residues 175 to 201 (bolded, SEQ ID NO:22) are normal *abl* sequence containing another primer binding site.

At page 48, lines 5-8:

The invention includes a [A] simplified method for preparing nucleic acid, preferably spliced mRNA, from a biological sample that is suitable for nucleic acid amplification [is disclosed]. The invention also includes a [A] method of detecting and measuring the amount of one or more species of spliced mRNA present in the sample, e.g., resulting from a *bcr-abl* fusion, following nucleic acid amplification of the spliced sequences [, is disclosed].

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APPENDIX B - Page 1

1. **(Amended Five Times)** A method for detecting a fusion nucleic acid [consisting essentially of] comprising the steps of:

a) providing a sample containing a first single-stranded fusion nucleic acid comprising a *bcr-abl* splice junction;

b) contacting under nucleic acid amplification conditions:

the first single-stranded fusion nucleic acid,

[a] an *abl*-specific first primer which hybridizes to [the fusion nucleic acid at] a first primer binding site of SEQ ID NO:22 [located 3' to the splice junction site], and

at least one nucleic acid polymerase activity;

c) amplifying the fusion nucleic acid in a single isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the *bcr-abl* splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site, and

a first probe binding site located [3' to] in a *bcr*-derived sequence and not overlapping the complementary splice junction site; [, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to the first primer binding site;]

d) hybridizing the second nucleic acid strands with [an] a *bcr*-specific oligonucleotide probe under hybridization conditions in which the probe hybridizes to [either] the first probe binding site [or the second probe binding site], thereby forming a probe:target hybrid; and

e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

2. (Reiterated) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is an mRNA, the first primer is a promoter-primer, the polymerase activity comprises an RNA polymerase activity, and the oligonucleotide probe is of the same sense as the mRNA and binds to the first probe binding site.

3. (**Amended**) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is a mRNA, wherein the second nucleic acid strands are complementary RNA, wherein the amplifying step includes contacting the second nucleic acid strand with a second primer or promoter-primer which hybridizes to a *bcr*-specific second primer binding site [located 3' to both the complementary splice junction and the first probe binding site] that hybridizes to SEQ ID NO:5, and wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity and an RNA-directed DNA polymerase activity.

4. (**Canceled**) The method of Claim 1, wherein the oligonucleotide probe binds to the second probe binding site and does not form a stable hybridization complex with the first single-stranded fusion nucleic acid.

5. (**Amended**) The method of Claim 1, [wherein the fusion nucleic acid is a *bcr-abl* fusion mRNA and] wherein the *bcr*-specific oligonucleotide probe has a sequence of SEQ ID NO:9 or SEQ ID NO:27. [binds to a *bcr*-derived nucleotide base sequence in the second nucleic acid strands.]

6. (Reiterated) The method of Claim 1, wherein step a) includes preparing RNA from the sample containing the fusion nucleic acid by:

contacting a biological sample comprising the fusion nucleic acid with a solution consisting essentially of:

a buffer,

about 150 mM to about 1 M of a soluble salt,

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and

a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms, directly or indirectly, a stable hybridization complex with an RNA under conditions permitting the formation of the stable hybridization complex; and

separating the hybridization complex joined to the solid support from unhybridized sample components without extracting the RNA using reagents such as phenol or chloroform.

7. (Reiterated) The method of Claim 6, wherein the fusion nucleic acid is mRNA.

8. (Reiterated) The method of Claim 7, wherein the nucleotide base sequence of the immobilized oligonucleotide comprises a poly-T sequence.

9. **(Amended Five Times)** A method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation [consisting essentially of] comprising the steps of:

a) providing a sample containing a fusion mRNA transcript comprising a *bcr-abl* splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

a first primer which hybridizes to a sequence of SEQ ID NO:22 [the fusion mRNA transcript at a first primer binding site derived from a first chromosomal region and located 3' to the splice junction site], and

at least one enzyme having nucleic acid polymerase activity;

c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses

the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the fusion mRNA transcript containing the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site derived from a *bcr* sequence [located 3' to] and not overlapping the complementary splice junction site, [wherein the first probe binding site is derived from a second chromosomal region,] and

a second probe binding site derived from an *abl* sequence [located 5' to] and not overlapping the complementary splice junction site, wherein the second probe binding site [is derived from a third chromosomal region and] overlaps or is located 3' to sequence complementary to SEQ ID NO:22 [the first primer binding site];

d) hybridizing the second nucleic acid strands with an oligonucleotide probe which hybridizes to the second nucleic acid strands at either the first probe binding site or the second probe binding site but does not hybridize to the fusion mRNA transcript, thereby forming a hybridization complex of the first probe or the second probe and the second nucleic acid strand; and

e) detecting the hybridization complex as an indication of the presence of the fusion transcript in the sample.

10. **(Amended)** The method of Claim 9, wherein the amplifying step uses only a first primer that is a promoter primer of SEQ ID NO:1 and the enzyme has an RNA polymerase activity, and wherein the hybridizing step uses an oligonucleotide probe which hybridizes to the second nucleic acid at the first probe binding site.

11. **(Canceled)** The method of Claim 9, wherein the first probe binding site and the second probe binding site are derived from different locations on the same chromosome in a eukaryotic cell, and the

fusion mRNA transcript detected results from an intrachromosomal translocation.

12. (Reiterated) The method of Claim 9, wherein the first probe binding site is derived from a different chromosome than the chromosome from which the second probe binding site is derived, and the fusion mRNA transcript detected results from a translocation involving different chromosomes.

13. **(Canceled)** The method of Claim 12, wherein the fusion mRNA transcript results from a translocation of human chromosomes selected from the group consisting of: t(1;19), t(2;5), t(2;13), t(4;11), t(6;9), t(8;21), t(9;11), t(9;22), t(11;14), t(11;19), t(11;22), t(12;21), t(14;18) and t(15;17) translocations.

14. **(Amended)** The method of Claim 9 [13], wherein the fusion mRNA transcript results from a human t(9;22) translocation [and the oligonucleotide probe comprises a *bcr* -derived sequence or an *abl*-derived sequence].

15. (Reiterated) One or more oligonucleotides suitable for use in the method of Claim 14, have a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:23, SEQ ID NO:26 and SEQ ID NO:27.

16. (Reiterated) The method of Claim 9, wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity, and an RNA-directed DNA polymerase activity, and further uses a second primer or promoter primer which hybridizes under amplification conditions to a nucleotide sequence of a complementary RNA produced during the amplifying step.

17. (Reiterated) The method of Claim 16, wherein the RNA-directed DNA polymerase activity and DNA-directed DNA polymerase activity are supplied by a reverse transcriptase.

18. (**Amended**) The method of Claim 9, wherein the amplifying step also amplifies an internal control normal *abl* transcript in the sample by using the first primer and then hybridizing [a] an *abl*-specific second oligonucleotide probe which hybridizes to the complement of the internal control transcript but does not hybridize to the complement of the fusion mRNA transcript thereby forming in internal control hybridization complex, and wherein the detecting step also detects the presence of the internal control hybridization complex in the sample, thereby providing an internal standard.

19. (Reiterated) A method of preparing a sample containing RNA suitable for amplification, consisting essentially of the steps of:

a) providing a biological sample comprising unpurified RNA;

b) mixing the biological sample with a solution consisting essentially of:

a buffer at a pH of about 6.5 to about 8.5,

about 150 mM to about 1M of a soluble salt, and

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, to produce a solution containing released RNA;

c) mixing the solution containing released RNA with a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions;

d) separating the hybridization complex joined to the solid support from unhybridized sample components; and

e) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

20. (Reiterated) The method of Claim 19, wherein the biological sample is uncoagulated blood,

plasma or bone marrow.

21. **(Amended)** A method of detecting a fusion mRNA transcript produced as a result of a human *bcr-abl* translocation comprising the steps of:

a) providing a sample containing a human fusion mRNA transcript comprising a *bcr-abl* splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

[a] an *abl*-specific first primer that binds to a primer binding site of SEQ ID NO:22, which [hybridizes to the a second primer which hybridizes to the complement of the fusion mRNA transcript at a primer binding site derived from a *bcr* region] is located in an *abl*-specific sequence flanking the *bcr-abl* splice junction site,

a *bcr*-specific second primer having a sequence of SEQ ID NO:5, which hybridizes to [the complement of the fusion mRNA transcript at a primer binding site derived from a *bcr* region] a *bcr*-specific sequence flanking the *bcr-abl* splice junction site,

at least one enzyme having an RNA-directed DNA polymerase activity, and

at least one enzyme having an DNA-directed RNA polymerase activity;

c) amplifying the fusion mRNA transcript in a single nucleic acid amplification reaction that uses

the *abl*-specific first primer [to produce a second nucleic acid strand complementary to at least a portion of the fusion mRNA transcript containing the *bcr-abl* splice junction site],

the *bcr*-specific second primer [to produce a third nucleic acid strand of the same sense as the fusion mRNA transcript containing the *bcr-abl* splice junction site], and

the DNA-dependent RNA polymerase activity to produce amplified RNA that is complementary to the fusion mRNA transcript comprising the *bcr-abl* splice junction;

d) hybridizing the amplified RNA with [an] a *bcr*-specific oligonucleotide probe which hybridizes to a probe binding site derived from a *bcr* region flanking the *bcr-abl* splice junction, thereby forming a hybridization complex; and

e) detecting the hybridization complex as an indication of the presence of the fusion mRNA transcript in the sample.

22. **(Amended)** The method of claim 21, further comprising

in the contacting step, contacting [a] an *abl*-specific third primer having a sequence of SEQ ID NO:13 that hybridizes to the complement of a normal *abl* mRNA transcript [at a site located downstream of the first primer in an *abl* sequence present in a normal *abl* mRNA transcript that is replaced by *bcr* sequence in the fusion mRNA transcript],

in the amplifying step, amplifying a normal *abl* sequence present in the normal *abl* mRNA by using the *abl*-specific first primer and the *abl*-specific third primer [that is replaced by *bcr* sequence in the fusion mRNA transcript],

in the hybridizing step, hybridizing an oligonucleotide probe that hybridizes to a probe binding site derived from an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, and

in the detecting step, detecting a hybridization complex made up of the probe hybridized to an *abl* sequence that is replaced by *bcr* sequence in the fusion mRNA transcript, thereby providing an internal control based on amplifying and detecting normal *abl* sequence.

23. **(Amended)** The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the *bcr*-specific oligonucleotide probe is of SEQ ID NO:9 or SEQ ID NO:27 or is a mixture of SEQ ID NO:9 [or] and SEQ ID NO:27.

24. (Reiterated) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second

primer is of SEQ ID NO:5 or its RNA equivalent, and the probe is of SEQ ID NO:9 or its RNA equivalent.

25. (Reiterated) The method of claim 21, wherein the first primer is of SEQ ID NO:1, the second primer is of SEQ ID NO:5, and the probe is of SEQ ID NO:27.

26. (Reiterated) The method of claim 22, wherein the third primer is of SEQ ID NO:13 or its RNA equivalent, and the oligonucleotide probe that hybridizes to the *abl* sequence is of SEQ ID NO:16 or its RNA equivalent or SEQ ID NO:26.